



## *Euwallacea perbrevis* (Schedl, 1951) and associated novel fungal symbiont, *Fusarium* sp.: A potential cause of wilting in cocoa, *Theobroma cacao* in India

Shivaji Hausrao Thube<sup>a,\*</sup>,<sup>1,2</sup>, R Thava Prakasa Pandian<sup>a,\*\*</sup>,<sup>1</sup>, M. Rajkumar<sup>b</sup>, Merin Babu<sup>c</sup>, A. Josephraj Kumar<sup>c</sup>, Santhoshkumar P<sup>a</sup>, B.J. Nirmal Kumar<sup>a</sup>, Vinayaka Hegde<sup>b</sup>, Balanagouda Patil<sup>d</sup>, H. Rajashekara<sup>e</sup>, T. Prabhulinga<sup>f</sup>, Babasaheb B. Fand<sup>f</sup>, Shailesh Gawande<sup>f</sup>, Dipak Nagrale<sup>f</sup>, M. Devindrappa<sup>f</sup>, M.K. Rajesh<sup>a</sup>

<sup>a</sup> ICAR-Central Plantation Crops Research Institute, Regional Station, Vittal, Karnataka, India

<sup>b</sup> ICAR-Central Plantation Crops Research Institute, Kasaragod, Kerala, India

<sup>c</sup> ICAR-Central Plantation Crops Research Institute, Regional Station, Kayamkulam, Kerala, India

<sup>d</sup> Department of Plant Pathology, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga, 577204, Karnataka, India

<sup>e</sup> ICAR-Directorate of Cashewnut Research, Puttur, 574202, Karnataka, India

<sup>f</sup> ICAR-Central Institute for Cotton Research, Nagpur, Maharashtra, India

### ARTICLE INFO

#### Keywords:

Cocoa  
Xyloborini  
*Fusarium solani* species complex  
Symbiosis  
Wilting

### ABSTRACT

Cocoa is a commercially important beverage crop that faces new challenges in the context of climate change, which can alter pest and disease dynamics in cocoa plantations. In the Dakshina Kannada district of Karnataka, India, an abrupt increase in cocoa wilt was observed during the 2017–18 and 2018–19 seasons. Systematic examination of affected trees revealed consistent attacks of *Euwallacea perbrevis* (Schedl, 1951; Coleoptera: Curculionidae: Scolytinae). In-depth studies of the wilted trees revealed the association of the plant pathogenic fungus *Fusarium* sp. with *Euwallacea perbrevis*. The identity of the obtained pure culture from the insect and infected tree was confirmed as *Fusarium* sp. using amplification of ribosomal DNA, RNA polymerase II largest subunit (*RPB1*) and translation elongation factor 1 (*Tef1*) genes. Notably, this study reports a remarkable case of mutualistic association between ambrosia beetles and *Fusarium* sp., belonging to the *Fusarium solani* species complex (FSSC), rather than the *Ambrosia Fusarium clade* (AFC). This finding highlights the intriguing diversity of mutualistic relationships in nature and sheds new light on the complex interactions between organisms. The present study also investigated the impact of rainfall on the colonization and spread of the *Euwallacea-Fusarium* complex. The findings revealed that rainfall was identified as a predisposing factor for the incidence and spread of attacks. Additionally, the study conducted a comprehensive analysis of the modeling of *Euwallacea-Fusarium* complex spread over a specific period of time.

### 1. Introduction

*Theobroma cacao* L., commonly known as cocoa, originated from South and Central America and has become a pivotal cash crop cultivated commercially across various humid tropical nations worldwide. The historical introduction of cocoa to India occurred in 1798 (Ratnam,

1961), marking the inception of its systematic cultivation as an intercrop or mixed crop in arecanut (*Areca catechu* L.), coconut (*Cocos nucifera* L.), and oil palm (*Elaeis guineensis* Jacq.) plantations within the southern states, specifically Karnataka, Kerala, Tamil Nadu, and Andhra Pradesh (Thube et al., 2022b). Presently, cocoa cultivation in India spans an extensive area of 97,563 ha, with an annual production reaching 25,783

\* Corresponding author.

\*\* Corresponding author.

E-mail addresses: [Shivaji.Thube@icar.gov.in](mailto:Shivaji.Thube@icar.gov.in) (S.H. Thube), [R.Pandian@icar.gov.in](mailto:R.Pandian@icar.gov.in) (R.T.P. Pandian).

<sup>1</sup> Both authors contributed equally.

<sup>2</sup> Present address: ICAR-Central Institute for Cotton Research, Nagpur, Maharashtra, India.

MT tonnes (DCCD, 2020). Despite the noteworthy domestic contributions, it is imperative to acknowledge that global cocoa production is heavily influenced by West Africa, which commands a substantial share of over 70% of the total output, with South-East Asia and Latin America closely following suit (Lahive et al., 2019).

Cocoa plays a crucial role in the chocolate industry and other confectionary products. Currently, there is a demand for 30,000 metric tonnes of dry cocoa beans per year in India. However, the country's domestic production falls short of meeting the expected demand, which results in a significant share of cocoa being imported from other countries that grow it (DCCD, 2020). Additionally, cocoa cultivation is threatened by several biotic and abiotic factors, with insect pests and diseases being a significant contributor to low production and productivity of cocoa not just in India, but also in other cocoa-growing regions (Thube et al., 2019).

The transformation of ambrosia beetles from ecological decomposers to economically important pests reflects a concerning trend driven by global trade and environmental changes. Historically, these beetles played a vital role in ecosystem processes by targeting already weakened trees. However, with the spread of invasive species and the evolution of symbiotic relationships that allow them to attack healthy trees, they have become a serious threat to forest health and economic stability. Research by [Batra \(1963\)](#) initially established the benign nature of ambrosia beetles as they primarily fed on decaying wood. Nonetheless, subsequent studies by [Kühnholz et al. \(2001\)](#), [Hulcr et al. \(2017\)](#) and [Thube et al. \(2022a, 2022b\)](#) have documented instances where certain species can infect healthy trees, leading to dieback or even tree mortality. This shift in behavior is facilitated by the symbiotic fungus carried by the beetles, which acts as a pathogen in healthy trees. The escalation of global trade, as highlighted by [Marini et al. \(2011\)](#), has accelerated the spread of invasive ambrosia beetle species. This proliferation poses a dual threat: ecological disruption to native ecosystems and economic harm to industries reliant on timber and healthy forests. The potential for significant economic damage, as noted by [Beaver \(1989\)](#) and further elaborated upon by [Hulcr and Stelinski \(2017\)](#), underscores the urgency of addressing the ambrosia beetle issue through comprehensive management strategies.

Xyleborini beetles rely on symbiotic microorganisms, mainly fungi, as their primary food source, which they carry with them in specific organs called mycangia ([Batra, 1963](#)). These symbiotic fungi are cultivated in galleries bored by the beetle into the host plant xylem ([Beaver, 1989](#)). However, in addition to providing food for the beetles, these fungi can also be harmful to the hosts they colonize, leading to its dieback or dead in the case of particularly virulent fungi ([Kühnholz et al., 2001](#); [Hulcr et al., 2017](#); [Hulcr and Stelinski 2017](#)). This is the case, for example, of the invasive *Xyleborus glabratus* Eichhoff, 1877 and its primary symbiont *Raffaelea lauricola* T.C. Harr., Fraedrich & Aghayeva, that upon their introduction in South-eastern USA killed over half a billion trees ([Hughes, 2013](#)).

*Euwallacea fornicatus* (Eichhoff, 1868) is a highly polyphagous ambrosia beetle ([Ruzzier et al., 2023](#)), primarily associated with its fungal symbiont *Neocosmospora euwallaceae* ([Freeman et al., 2013](#)). This beetle of Oriental origin is now established in Florida, USA ([Carrillo et al., 2016](#)), California, USA ([Eskalen et al., 2012](#)), Israel ([Mendel et al., 2012](#)), South Africa ([Paap et al., 2018](#)) and Argentina ([Ceriani-Nakamurakare et al. 2022](#)), where it poses a threat to many fruits and forests trees ([Haack and Rabaglia, 2013](#)).

Despite damages caused by Xyleborini were historically recorded on *Theobroma cacao* ([Prior, 1986](#); [Brayford, 1987](#)), no substantial attacks by *E. perbrevis* was documented along both its native and invaded range prior to this publication. Beginning in 2017–18, substantial wilting was observed in cocoa plantations in Karnataka, India, imputable to primary attacks by *E. perbrevis*. Preliminary investigations confirmed the identity of the beetle but showed that the pathogenic fungus associated with it was not the most commonly known symbiont, but a different species. These results are presented and discussed in this article, in association

with an assessment of the incidence of attacks and triggering causes.

## 2. Materials and methods

### 2.1. Study site and sample collection

Thirteen-year-old cocoa trees ( $n = 5$ ) showing signs of decline with small pinholes on the stem were selected from the research field of ICAR-Central Plantation Crops Research Institute, Regional Station, Vittal, Karnataka (12.45° N latitude, 75.4° E longitude and 90 m above MSL). All the internal and external symptoms were recorded systematically by following destructive sampling of all the infested trees. Insect specimens and fresh discolored wood samples with insect galleries were collected for further investigations.

### 2.2. Ambrosia beetle identification

Adult specimens identified *via* morphological criteria were further subjected to molecular characterization using the mitochondrial cytochrome *c* oxidase I (*mt COI*) gene using LCO1490 and HCO2198 primers ([Folmer et al., 1994](#)). Except for the annealing temperature, which was set to 53 °C, all the other steps related to PCR amplification, gel visualization, and sequencing were carried out in the same manner as described in the pathogen characterization. Obtained sequences were aligned using BioEdit (Biological sequence alignment editor-Tom Hall, <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>) and blasted in BOLD database (<http://www.boldsystems.org/>). Sequence similarity was analyzed with the available sequences and sequences were deposited in NCBI.

### 2.3. Pathogen isolation

Fungal isolates used under present investigations were obtained from the heads of female beetles following the protocol described by [Lynch et al. \(2016\)](#) and from insect galleries of cocoa wood similar to the method described by [Eskalen et al. \(2013\)](#). The 20 adults were initially surface sterilized using 70% ethanol. After evaporation of ethanol, samples were washed with 0.3% Sodium hypochlorite (NaClO) solution for avoiding the chances of external contamination and allowed to dry on sterile filter paper. Beetle heads were dissected out from the whole body under a stereomicroscope (Leica M10, equipped with an EC4 Digital camera). All the 20 head segments were crushed individually in 1.5 ml microcentrifuge tubes with sterile micro-pestle. The macerated heads were separately suspended in 200 µl of sterile distilled water and 30 µl of the suspensions were pipette out on Petri-plates containing potato dextrose agar (PDA) amended with 0.01% (w/v) Rifampicin and spread using sterile L-shaped glass rod. Plates were incubated at 25–30°C and single spore fungal colonies with unique morphologies were sub-cultured for further identification. Likewise, 20 fungal isolates from *Euwallacea* were maintained at Plant Pathology laboratory of Central Plantation Crops Research Institute Regional Station, Vittal for further experiments.

Simultaneously, 20 numbers of insect galleries were surface sterilized using 0.4% sodium hypochlorite (NaClO) solution for 5 min and divided into small pieces. All the pieces of the galleries were inoculated separately on PDA-r medium for single spore isolation of the pathogen. Inoculated Petri-plates were incubated at 25–30°C and single spore fungal colonies with unique morphologies were sub-cultured for further identification.

Fungal cultures obtained from insect specimens and gallery samples were compared and characterized for the proper identity of fungus.

### 2.4. Characterization of pathogen

Cultural and microscopic characterization (Nikon Eclipse Ni-U, Tokyo, Japan) of 10 days old fungus culture was carried out by using the

taxonomic keys given by Nelson et al. (1983). A small actively growing mycelial disc (2–5 mm) of the fungus was inoculated into a conical flask containing 100 ml potato dextrose broth (PDB). Fungal mycelium was filtered by using sterile No. 1 Whatman filter paper (HIMEDIA Labs, India). The mycelial mat was ground with liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . Total fungal DNA was extracted from 1 g freeze-dried samples by the CTAB method with minor modifications (Pandian et al., 2022). Multilocus sequence typing (MLST) was performed to amplify the internal transcribed spacer region of ribosomal DNA (rDNA) with ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGTTAT TGATATGC-3') (White et al., 1990), translation elongation factor 1- $\alpha$  (Tef 1- $\alpha$ ) with EF1F (5'-ATGGGTAAGGARGACAAGAC-3') and EF1R (5'-GGARGTACCAGTSATCATG-3') (O'Donnell et al., 2015), DNA-directed RNA polymerase II largest subunit (RPB1) with RPB1F (5'-GAACGGTGTCAGCAAGTTGTG-3') and RPB1R (5'-GACTGMGC AGCCAGAACACCA-3') (Pandian et al., 2022). PCR reaction was performed for each gene with a 50  $\mu\text{l}$  reaction volume containing 5.0  $\mu\text{l}$  of 10X PCR buffer (Thermo Fisher Scientific, USA), 2.0  $\mu\text{l}$  of 10 mM dNTPs, 2.0  $\mu\text{l}$  each of forward and reverse primers (10 pmol/ml), 2.0  $\mu\text{l}$  of DNA, 1.0  $\mu\text{l}$  of Taq DNA polymerase, and 36.0  $\mu\text{l}$  of sterile water. Thermocycling conditions were optimized for each gene. For the ITS region, the PCR conditions included initial denaturation at  $95^{\circ}\text{C}$  for 5 min, followed by 30 cycles of denaturation at  $95^{\circ}\text{C}$  for 30 s, annealing at  $50^{\circ}\text{C}$  for 30 s, extension at  $72^{\circ}\text{C}$  for 30 s, and final extension at  $72^{\circ}\text{C}$  for 5 min. For RPB1, TEF1, and mt COI genes, annealing temperatures of  $54^{\circ}\text{C}$ ,  $60^{\circ}\text{C}$ , and  $53^{\circ}\text{C}$ , respectively, were used, while other steps remained the same.

The amplified products were evaluated using a 1.0% agarose gel and electrophoresis (Best Lab International Inc., China). The PCR products were sent for Sanger's sequencing (Biokart India Pvt. Ltd., Bengaluru, India). The obtained sequences were aligned using BioEdit and blasted in the NCBI. Sequence similarity was analyzed with the available sequences, and the sequences were deposited in NCBI.

## 2.5. Pathogenicity test

In order to fulfil the Koch's postulate, pathogenicity assay was conducted using 30 cm long healthy cocoa shoot detached from thirteen-year-old tree under controlled laboratory conditions. Xylem tissues were excised from two sites on each stem, spaced 10 cm apart (Total  $n = 5$  shoots), with a mean diameter of  $10.5 \text{ mm} \pm 0.3 \text{ mm}$ , using a 7 mm cork borer. We inoculated each isolate 10 times in this manner. A 7 mm diameter of seven days old FSSC culture (CPCRI\_FSSC\_Fusarium sp. *Euwallacea* and CPCRI\_FSSC\_Fusarium sp. *Cocoa*) was taken from the periphery, placed onto the freshly wounded tissue with the mycelium facing down, and wrapped with parafilm. Plain agar discs were used as control. The inoculated stems ( $n = 5$  each) were incubated in a moist chamber at  $25 \pm 2^{\circ}\text{C}$  for 15 days. The vertical and horizontal spread of vascular discoloration was measured and re-isolation was done from the symptomatic tissues. The experiment was carried out in a completely randomized design (CRD) with 10 inoculations per isolate and the experiment was repeated twice.

The pathogenicity of the monoconidial culture was further evaluated on a 13-year-old cocoa tree under field conditions. A 7 mm-sized fresh tissue was excised at 1 cm depth from healthy cocoa stems/branches using a sterile cork borer. Mycelial disc of 10 days old monoconidial culture was inoculated in each wounded site. Inoculated discs were covered with a fresh sterile cocoa wood disc of 5 mm size. Inoculated sites were covered with cotton moistened with sterile water and fastened with a parafilm sheet (Supplementary Fig. 1.). The area of vascular discoloration was measured, and after 25 days of inoculation, re-isolation was performed from the symptomatic tissues. A PDA disc without monoconidial culture was inoculated as a control.

## 2.6. Effect of rainfall on colonization of *Euwallacea-Fusarium* complex

Since populations of bark and ambrosia beetles and associated fungi

respond to climatic variation (Ranger et al., 2012, 2013; Gomez et al., 2018; Sittichaya et al., 2012), the daily values of rainfall and relative humidity were collected from the experimental site to test whether these variables explain the colonization of *Euwallacea-Fusarium* complex in cocoa.

Observations on percent colonization of the *E. perbrevis-Fusarium* complex was recorded from 15-year-old cocoa plantation of 1 ha (Upper Amazon cocoa genotypes) located at the Indian Council of Agricultural Research—Central Plantation Crops Research Institute Regional Station, Vittal (ICAR-CPCRI-RS, Vittal), Karnataka, India ( $12.45^{\circ}\text{N}$ ,  $75.4^{\circ}\text{E}$ ; 90 MASL) during the first week of August for six consecutive years (2016–2021). Cocoa stems/branches at the early stages of beetle colonization were identified by observing the extrusion of wood noodles. Identity of the *Euwallacea* and *Fusarium* from newly colonized trees was confirmed every year by random sampling of insect and fungal isolates. Percent colonization of *E. perbrevis-Fusarium* complex was calculated as;

$$\% \text{ Colonization} = \text{Number of symptomatic trees} \div 650 \times 100$$

**Note:** '650' are number of trees per hectare area.

However, the percent spread of *E. perbrevis-Fusarium* complex was calculated as;

$$\% \text{ Spread} = (\% \text{ colonization in respective year} - \% \text{ colonization in first years})$$

The impact of rainfall on the progress of *Euwallacea-Fusarium* was assessed by regressing the spread (%) of this complex against the cumulative annual rainfall for each year. Commonly used statistical measures, such as the coefficient of determination ( $R^2$ ), residual patterns, and root mean square errors (RMSE), were employed to evaluate the suitability of the significant models. The following linear model equations were utilized to examine the effect of rainfall on the progress of disease development.

$$Y = a \pm bX \quad (3)$$

Where, 'Y' is the disease progress (%), 'X' is the cumulative annual rainfall (mm), and a and, b are the model parameters.

## 2.7. Modeling the spread of *Euwallacea-Fusarium* complex

To model the progression of *Euwallacea-Fusarium* complex spread over a six-year period (2016–2021), we regressed the disease infection severity values (%) observed each year against the corresponding years. The Logistic and Gompertz models were employed for this analysis. The intercepts and coefficients of the resulting models were compared using an F-test ( $p \leq 0.05\%$ ). The following equations were utilized: one for the Logistic model and the other for the modified Gompertz model.

$$\text{Disease spread (\%)} = \frac{C}{1 + \lambda * \exp(-\mu m * t)} \quad (1)$$

$$\text{Disease spread (\%)} = c * \exp\left\{-\exp\left[\frac{\mu m * e}{C} * (\lambda - t) + 1\right]\right\} \quad (2)$$

Where, 'c' is the asymptote towards which disease is progressing, ' $\mu m$ ' is the maximum rate of progress of disease spread (%) over time 't' i.e.  $\ln\left(\frac{N_{max} - N_0}{n}\right)$ , ' $\lambda$ ' is the time elapsed until the  $\mu m$  is reached. i.e.  $\ln\left(\frac{N_{max}}{N_0} - 1\right)$ , 'e' is the Euler's number; whose value is approximately equal to 2.7183

## 3. Results

### 3.1. Symptoms of infestation

The symptoms of the infestation recorded during the study were as

follows; yellowing of the leaves, which was noted during the initial stage of the infestation (Fig. 1a), extrusion of wood powder in the form of loose, cylindrical strings from the infested bark (Fig. 1b), presence of round holes, measuring between 1.2 and 1.80 mm, under the bark of the infested tree (Fig. 1c), numerous galleries were found, ranging from 1.3 to 1.42 mm in diameter, with a large number of immature stages and adult beetles (Fig. 1d), vascular discoloration was observed in the infested heartwood (Fig. 1e) and drying of infested trees was evident during the advanced stage of infestation. These symptoms collectively illustrate the progression and impact of the infestation on the affected trees.

### 3.2. Insect species identification

The partial *mt CO1* gene sequence (569 bp) of the adult beetle was deposited in the GenBank (Accession number MT066045). The homology sequence analysis revealed 99.29 % sequence similarity with those of *Euwallacea fornicatus* (Later changed to *E.perbrevis* as per Smith et al., 2019) *mt CO1* gene sequences available in NCBI with Accession No. KX371815 and KJ866509. As elucidated from the phylogenetic tree constructed using *mt CO1* gene sequences, *E. fornicatus* (= *E.perbrevis* under present study and as per Smith et al., 2019) infesting cocoa formed a monophyletic clade with all the species belonging to clade TSHBa given by Gomez et al. (2018) (Fig. 2).

### 3.3. Fungus identification

An isolated fungus (from adult beetles and infected parts) produced

aerial, whitish to orange-colored, cottony mycelium on the upper side of PDA plates (Fig. 3a). Microscopic observations recorded macroconidia that were hyaline, two-septate, sparse, and produced occasionally (Fig. 3b). Meanwhile, microconidia were abundant, zero-to single-septate, oval-to elongated-oval-shaped, with a size of  $8.47 \pm 0.20 \times 2.59 \pm 0.07 \mu\text{m}$  (Fig. 3c). Chlamydoconidia formed relatively abundantly in the mycelium, mostly globose to subglobose, intercalary, and rough (Fig. 3d). Cultural and microscopic features revealed the isolated pathogen as a member of the *Fusarium solani* species complex (FSSC) (Booth, 1971). Fungal isolates obtained from *Euwallacea* and cocoa wood were named as CPCRI\_FSSC\_ *Fusarium* sp. *Euwallacea* and CPCRI\_FSSC\_ *Fusarium* sp. *Cocoa* respectively. The reference isolates are maintained in the Plant Pathology Repository, ICAR-CPCRI, Regional Station, Vittal, Karnataka, India.

FSSC partial gene sequence of *ribosomal* DNA (568 bp), *tef1* (624 bp) and *RPB1* (984 bp) were deposited in GenBank with accession numbers MT107069-MT107081, MT119145- MT119148, and MW815606 respectively. Earlier studies have demonstrated that *RPB I* and *RPB II* are more reliable for the proper identification of species belonging to the *Fusarium* genus (Akbar et al., 2018). A BLAST analysis of the *RPB I* sequences against the NCBI database revealed a 99.20% identity to a *Fusarium* sp. known for causing big-leaf disease of Mahogany in Mexico (Accession number: MG838042). Based on the aforementioned results, these isolates were considered members of the genus *Fusarium* belonging to the FSSC.

To establish the phylogenetic relationship with other species from the FSSC, a multilocus sequence analysis was performed using the *ITS*, *TEF 1* and *RBP 1* sequences. The analysis revealed that the fungal isolates



**Fig. 1.** Infestation symptoms of the *Euwallacea-Fusarium* complex: a-Initial yellowing of infested tree; b-frass nodules emission; c- Presence of pinhead-sized round emergence holes; d-the presence of insect galleries with large numbers of immature stages and adult beetles; e-vascular discoloration of infested heartwood; f-drying of infested trees.

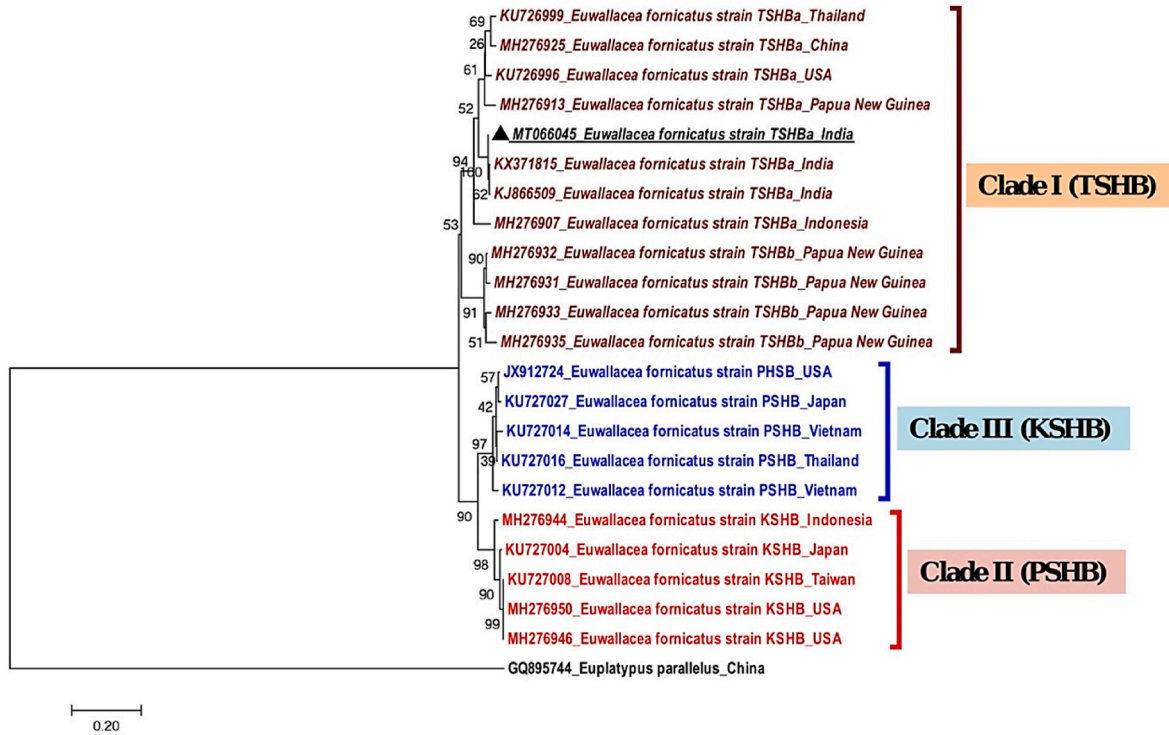


Fig. 2. Phylogenetic tree and molecular lineage of *Euwallacea fornicatus* (Clade TSHB= *E. perbrevis*) with other species belong to various established clades of *Euwallacea fornicatus* species complex based on *COI* gene using *Euplatypus parallelus* as out-group taxa.

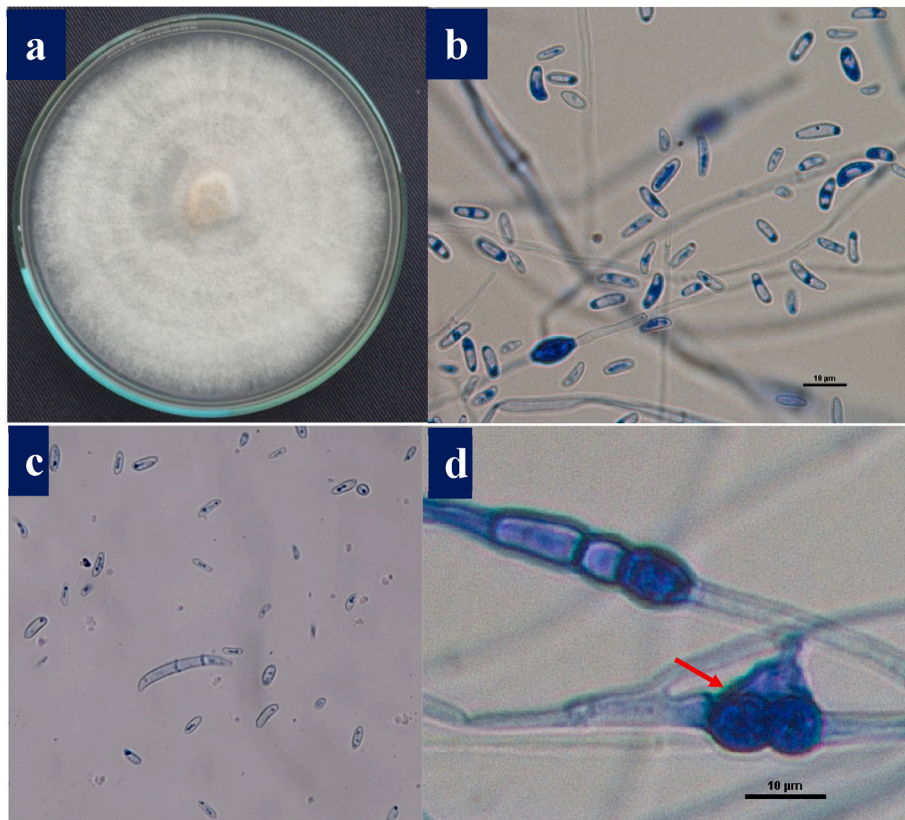


Fig. 3. Cultural characteristics of CPCRI\_FSSC\_Fusarium sp. (Isolated from *Euwallacea* beetle); a. Mycelial growth on PDA media, b. Abundant single celled microconidia, c. Sparse two septate macroconidia, d. Globose intercalary chlamydospores.

identified in this study belong to clade 3 but not to AFC, which consists of *F. ambrosium* AF-1, *F. euwallaceae* AF-2, *F. oligoseptatum* AF-4, *F. tuaranense* AF-5, *F. obliqueseptatum* AF-7, and *F. kuroshium* AF-12 (Fig. 4). However, all four isolates formed a highly supported clade, indicating their close relatedness to each other and their relationship to the clade represented by *F. spathulatum* NRRL 28541 (FSSC 26) and *F. ferrugineum* NRRL 32427 (FSSC 28).

### 3.4. Pathogenicity

All of the inoculated stems exhibited symptoms, and the fungus colonized the healthy *T. cacao* stems under in-vitro conditions. The pure culture was retrieved from all of the inoculated stems. There were no significant differences in lesion length among the replications. Horizontal lesion spread was measured as  $2.68 \pm 0.21$  cm in circumference seven days post-inoculation (dpi) (Fig. 5a). However, vertical spread was recorded at a depth of  $3.4 \pm 0.54$  cm from the point of inoculation (Fig. 5b). All of the inoculated cocoa branches exhibited vascular discoloration surrounding the site of inoculation under field conditions after 25 dpi (Fig. 5c).

### 3.5. Effect of rainfall on incidence and spread of *Euwallacea-Fusarium* complex

The current study analyzed the impact of total annual rainfall on incidence and spread of the *Euwallacea-Fusarium* complex in *T. cacao* plantations. The analysis spanning six years (2016–2021) revealed a significant positive correlation between the incidence and/or spread of

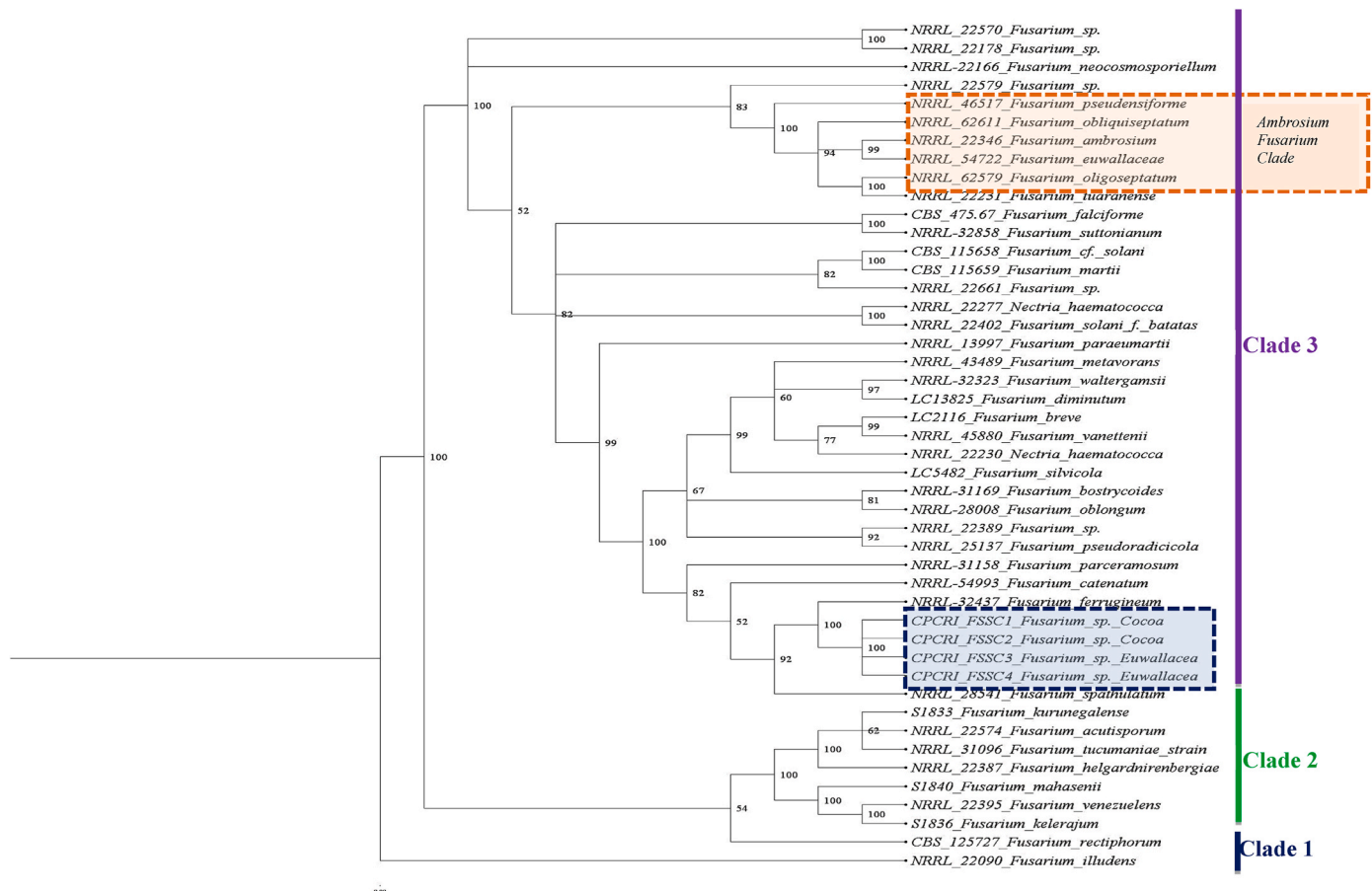
this complex with total annual rainfall. The fitting of the linear regression equation provided a good fit to the data on *Euwallacea-Fusarium* colonization in response to the annual total rainfall received during 2016–2021 ( $R^2 = 0.82$ ,  $P = 0.01$ ) (Table 1). The steady increase in the amount of annual rainfall received during the six-year study period was found to favor the progress and spread of the infestation (Table 2). The maximum spread (13.49%) was noticed in the year 2019, which coincided with the maximum annual rainfall of 4148.2 mm among all the years of the study period.

### 3.6. Modeling the spread of *Euwallacea-Fusarium* complex

The data on the spread of the *Euwallacea-Fusarium* complex over a period of six years (2016–2021) fit both the Gompertz ( $R^2 = 0.998$ , RMSE = 0.82) and Logistic ( $R^2 = 0.997$ , RMSE = 0.83) growth models (Table 3). The lower RMSE value, higher  $R^2$  value, and the pattern of residuals obtained for the Gompertz model indicated a lower standard deviation between actual observations and predicted values (Table 4).

## 4. Discussion

*Euwallacea fornicatus* is a pest that has gained increasing importance worldwide due to its ability to damage a wide range of economically important plants, particularly trees in urban and agricultural areas. The data and observations provided here confirm the pathogenicity of the beetle and its symbiont along its native range, and specifically for non-native plants cultivated for production, such as *T. cacao*. This pest is native to Southeast Asia but has now spread to many other regions of the



**Fig. 4.** Cladogram of the phylogenetic relationship of *Fusarium* sp. CPCRI\_FSSC1-4 with members of the three clades of FSSC. Phylogeny constructed through Bayesian inference using a combined data set of three gene markers' (*tef 1*, *ITS*, and the largest subunit of RNA polymerase (*RPB1*)) sequences. The representative species of clades I, II and III are those proposed by O'Donnell (2000). The AFC is highlighted in brown. Support values from the Bayesian inference are indicated at the nodes. CPCRI isolates are highlighted blue.

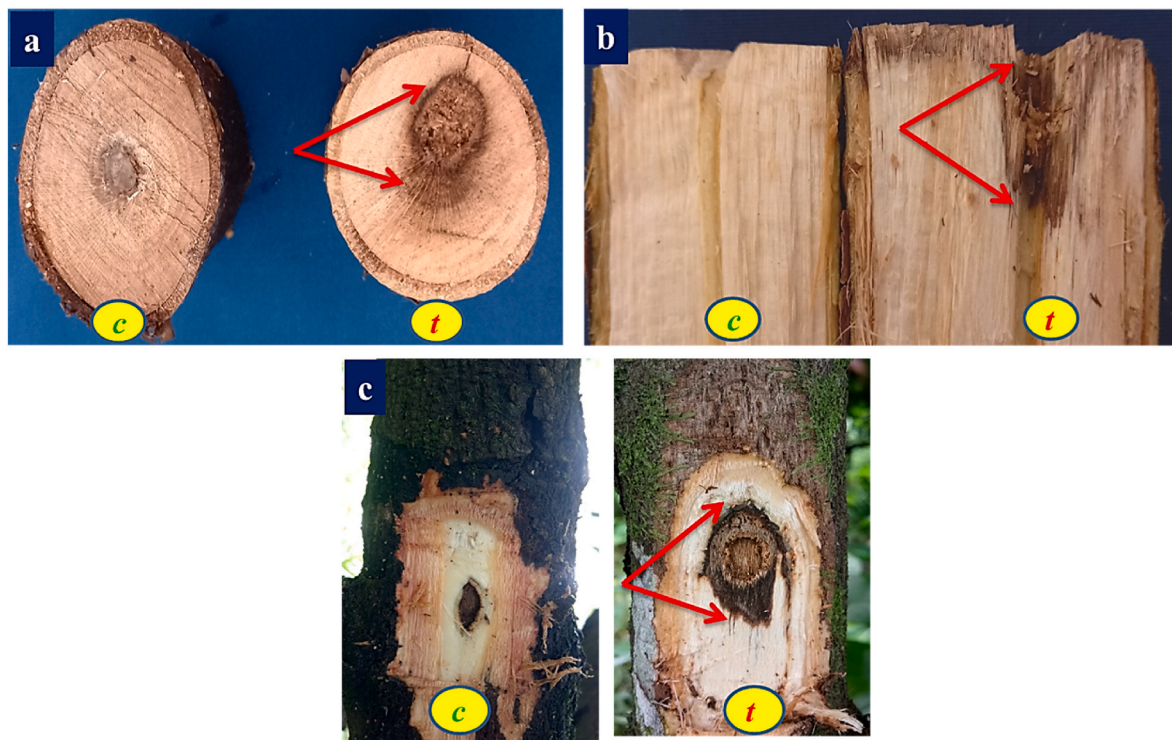


Fig. 5. Symptom expression of *Fusarium* sp., a-b. Horizontal and vertical spread of symptoms under laboratory conditions, c. symptom visualization under field conditions

Note: “c” and “t” at the bottom of all images represent control and treatment, respectively.

Table 1

Parameter estimates of linear regression equation fitted between cumulative annual rainfall and *Euwallacea-Fusarium* colonization over the time.

Intercept (a)	Slope (b)	df	F	R <sup>2</sup>	p value
-5.28 ± 3.08	0.004 ± 0.00	1,4	18.63	0.82	0.01

world, including Africa, Europe, North America, South America, and Oceania (Stouthamer et al., 2017).

Stouthamer et al. (2017) conducted a thorough phylogenetic assessment, identifying three primary genetic clades within the *E. fornicatus* species complex. These clades exhibited consistent correlations with phylogeographic patterns in both native and invaded regions. The lineage recognized as *E. fornicatus sensu stricto* (commonly referred to as Tea Shot Hole Borer or TSHB-Clade I) originates from Southeast Asia and was subsequently introduced into the United States. Another lineage, known as Kuroshio Shot Hole Borer or KSHB-Clade II, originated in Taiwan and Japan, later finding introduction into San Diego County in California and Mexico (García-Avila et al., 2016). The third lineage, likely originating from Southeast Asia, was introduced into Los Angeles County, California (United States), as well as Israel and South Africa (Stouthamer et al., 2017; Paap et al., 2018), earning the common name Polyphagous Shot Hole Borer (PSHB-Clade III) due to its wide host range. Building on the work of Stouthamer et al. (2017), Gomez et al. (2018) not only confirmed the three genetic lineages within the *E. fornicatus* species complex but also presented compelling evidence for a fourth lineage. The TSHB clade, as per the phylogenetic tree and average pairwise distances, was further subdivided into TSHBa (corresponding to clade 1B in Stouthamer et al., 2017) and TSHBb (corresponding to clade 1A in Stouthamer et al., 2017). In a recent taxonomic revision by Smith et al. (2019), based on the rediscovered syntype of *Xyleborus fornicatus*, the “tea shot hole borer clade a” was reclassified as *E. perbrevis*. The current phylogenetic analysis aligns with the assessments conducted by Stouthamer et al. (2017), Gomez et al. (2018) and

Smith et al. (2019). Specifically, the constructed phylogram using COI sequences reveals that *Euwallacea* species infesting cocoa forms a monophyletic clade with other *Euwallacea* species belonging to TSHB-Clade I, particularly with species from subclade TSHBa (Fig. 2). Therefore, although the BLAST analysis of nucleotide sequences identified the present species as *E. fornicatus*, the exact identity of this species is *E. perbrevis*.

One of the primary ways in which *E. fornicatus* causes damage is by transmitting *Fusarium* dieback, a disease caused by *Fusarium* fungi that can cause wilting, dieback, and ultimately, the death of infected trees (Hulcr et al., 2019). The beetles bore into the wood of trees, where they create galleries or tunnels for the fungal growth. As the fungus grows, it forms a mat of mycelium that the beetles feed on, providing them with a source of nutrition. In return, the beetles provide a suitable environment for the fungi to grow and reproduce (Biedermann and Vega, 2020). The relationship between *E. perbrevis* and *Fusarium* fungi is indeed fascinating, albeit destructive for the host trees. This mutualistic symbiosis showcases how intricate ecological interactions can be, even when they lead to detrimental outcomes for other organisms, in this case, the trees affected by *Fusarium* dieback disease (Eskalen et al., 2013). The specialized organs called mycangia, found in female *E. fornicatus* beetles, play a crucial role in the dissemination of *Fusarium* between host trees. These beetles essentially act as vectors for the fungus, aiding its spread while simultaneously benefiting from it as their primary food source.

The *Fusarium solani* species complex encompasses various fungal species, some of which have been identified as phytopathogens capable of causing significant damage to host trees (Na et al., 2018). By obstructing the vascular transport system of the trees, these fungi disrupt the flow of water and nutrients, leading to symptoms like wilting, dieback, and ultimately, the demise of the tree (Ploetz et al., 2013). Despite the destructive nature of this relationship for the trees, the association between ambrosia beetles and *Fusarium* fungi is often regarded as a mutualistic symbiosis (Hulcr and Dunn, 2011). The beetles derive nourishment from the fungus, while the fungus gains dispersal through

**Table 2**

The effect of cumulative annual rainfall on progress of *Euwallacea-Fusarium* colonization as predicted by linear regression model.

Year	Cumulative annual rainfall (mm)	Disease spread (%)		Residuals
		Observed value	Predicted value	
2016	1656.4	1.54	1.23	0.31
2017	2928.7	5.00	6.23	-1.23
2018	4120.8	8.55	10.91	-2.36
2019	4148.2	13.49	11.02	2.47
2020	3554.3	8.52	8.69	-0.16
2021	3235.6	8.41	7.43	0.97

**Table 3**

Parameter estimates of Gompertz and Logistic equations fitted for estimating the infestation progress over the period.

Model	C	$\lambda$	$\mu$	df	RMSE	R <sup>2</sup>
Gompertz	45.34	0.48	10.06	3,3	0.82	0.998
Logistic	39.55	16.34	1.12	3,3	0.83	0.997

the beetles and access to nutrients and protection. This intricate interdependence highlights the complexity of ecological systems and the often-unexpected consequences of seemingly innocuous interactions.

In India, invasive bark and ambrosia beetles pose a serious threat to the forest ecosystem, especially plantation crops (Thube et al. 2019, 2022a, 2022b). Fungal species as mutualistic companions of bark ambrosia beetles in aggravating tree diseases are reported worldwide (Thube et al., 2022a, 2022b; Harrington et al., 2014). Hence, it is imperative to understand the role of the associated fungi in pathogenicity and disease development. The present study is of great importance as it is the first study to investigate the mutualistic association between *Euwallacea perbrevis* and *Fusarium* infesting cocoa in India. Despite the significant economic importance of cocoa, little is known about the interactions between ambrosia beetles and their fungal mutualists in cocoa plantations. This study has confirmed the association of the novel strain of *Fusarium* spp. with the ambrosia beetle *E. perbrevis*, as evidenced by the isolation of fungal colonies from female beetles.

In India, the association of *E. fornicatus* with *Fusarium ambrosium* was documented in 1987 (Brayford 1987; Kasson et al., 2013). Ambrosia beetles, which are part of the *E. fornicatus* species complex, native to Southeast Asia (Beaver, 1989; Hulcr and Stelinski, 2017) and have been recognized for establishing a distinct mutualistic relationship with *Fusarium* species within the *Ambrosia Fusaria clade* (AFC) (Kasson et al., 2013; O'Donnell et al., 2015). Beyond their connections with AFC members, ambrosia beetles belonging to the *Euwallacea* genus have also been documented to form associations with various other fungal genera. These include *Graphium* spp. (Beaver, 1989; Freeman et al., 2016; Kasson et al., 2013; Lynch et al., 2016), *Paracremonium* spp. (Lynch et al., 2016), *Raffaelea subfusca* (Kasson et al., 2013), *Acremonium* sp., *Acremonium morum*, *Acremonium masseei*, and *Elaphocordyceps* sp. (Carrillo et al., 2016).

The *Fusarium* genus, along with members of the FSSC, is established as a consistent partner of ambrosia beetles globally (Bateman et al., 2016). It has been documented not only in the Circeo National Park (Vannini et al., 2017) and Southern Italy (Bosso et al., 2012) but also in the USA and Japan (Bateman et al., 2016), having been isolated from *X. compactus*. *Fusarium*, known for causing serious tree diseases such as bark and wood cankers like *Fusarium circinatum* on pine (Wingfield et al., 2008) and vascular wilt such as *F. oxysporum* f. sp. *albedinis* on palms (Tantaoui et al., 1996), is recognized as a significant tree pathogen. Additionally, studies have indicated that members of FSSC isolated from *X. compactus* galleries and adjacent black-stained woody tissues exhibit high pathogenicity to *Quercus ilex* in Southern Italy (Bosso et al., 2012). Morales-Rodríguez et al. (2021) documented a severe infestation of the alien ambrosia beetle *X. compactus* on three Mediterranean woody hosts,

**Table 4**

The progress of *Euwallacea-Fusarium* spread over time as predicted by Gompertz and Logistic models.

Year	Disease spread (%)				
	Observed value	Predicted value		Residuals	
		Gompertz model	Logistic model	Gompertz model	Logistic model
2016	1.54	1.21	2.28	0.33	-0.74
2017	6.46	6.23	6.27	0.23	0.19
2018	14.46	15.31	14.52	-0.85	-0.06
2019	26.00	25.03	25.36	0.97	0.64
2020	32.31	32.76	33.47	-0.45	-1.16
2021	38.00	37.95	37.35	0.05	0.65

*Q. ilex*, *Laurus nobilis*, and *Ceratonia siliqua* in the Central Italy. This infestation resulted in wilting and necrosis of terminal branches in infested trees and shrubs, attributed to the combined impact of the insect and associated pathogenic fungi. Subsequent investigations revealed the association of various fungal taxa with diverse life strategies, including true mutualists like *Ambrosiella xylobori* and plant pathogens such as *Fusarium* spp.

Multigene molecular analyses conducted under present study have discovered a new strain of *Fusarium* belonging to the *Fusarium solani* species complex (FSSC), which is genetically distinct from the *Fusarium* species of the *Ambrosia Fusarium clade* (AFC), in Indian tea shot-hole borer (TSHB) population infesting cocoa. The study's results are consistent with the findings of Carreras-Villasenor et al. (2022), who also reported a mutualistic association between *Fusarium* isolates and the ambrosia beetle *Xylosandrus morigerus*, which belongs to the *Fusarium solani* species complex (FSSC) but not to the *Ambrosia Fusarium clade* (AFC). The *Fusarium* strain associated with TSHB is shown to be pathogenic to cocoa under both laboratory and field conditions. *Fusarium solani* species complex (FSSC) is a group of genetically diverse fungal species that can cause a range of diseases in trees, including root rot, damping-off, canker, and wilt. These diseases can lead to tree mortality, reduced tree growth, and decreased forest health. In general, forests trees are more prone to FSSC due to their high density and diversity, high levels of organic matter, natural disturbances, and human activities such as logging and land use change. The study site for the present investigation is located at the research field of ICAR-Central Plantation Crops Research Institute, Regional Station in Vittal, Karnataka (12.45° N latitude, 75.4° E longitude, and 90 m above MSL). The field is surrounded by forest plantations and is adjacent to the Western Ghats hills of South India, which is known to be a biodiversity hotspot. The population of *E. perbrevis* might have originally evolved/adopted with a novel mutualistic strain of FSSC in an unexplored and undisturbed forest ecosystem, and then shifted to adjacent cocoa plantations. Moreover, *F. solani* is reported to be associated with sudden death of *T. cacao* in Papua New Guinea (Prior, 1986). Hence, another probable reason for this strong association might be the contamination of adult beetles' mycangia with the identified *Fusarium* strain, which could have originally been a cocoa plant pathogen (Rosmana et al., 2013).

Our findings about impact of rainfall on establishment and spread of *Euwallacea-Fusarium* complex support the existing research indicating that the stress caused by flooding or water stagnation plays a crucial role in facilitating the colonization of ambrosia beetles by the *Fusarium* fungus (Ranger et al., 2013). The high incidence under elevated humidity is recorded due to the accelerated seasonal flight pattern of ambrosia beetles (Sittichaya et al., 2012). Likewise, sudden emergence/shifting of *Euwallacea* beetle to cocoa might be due to the accelerated seasonal flight patterns of ambrosia beetles under stressed condition caused due to submergence of field under water. Our data shows an annual trend of disease complex spread in response to annual total rainfall. The Gompertz model provided slightly better prediction of disease complex development and spread than did the Logistic model.

The superiority of the Gompertz model over the Logistic model in predicting the disease development and progress has been widely recognized (Bowen et al., 2018).

The findings from this study not only contribute to the understanding of the diversity of *Fusarium* species associated with *E. perbrevis* but also shed light on the potential impact of this association on cocoa production. Furthermore, we conducted a comprehensive analysis of the modeling of *Euwallacea-Fusarium* spread over a specific period of time. Our analysis revealed the relationship between the annual trend of disease complex spread and annual total rainfall.

Moreover, we recognized the importance of future studies focused on investigating the seasonal dynamics of the disease complex in response to various weather factors. Such studies would provide deeper insights into the onset and peaks of this complex, facilitating the development of targeted management strategies. This information is crucial for effectively controlling the spread of *E. perbrevis* and its associated fungi in cocoa plantations. By implementing these management strategies, we can help reduce economic losses for farmers and ensure the sustainability of cocoa production in the face of potential challenges posed by the *Euwallacea-Fusarium* complex.

### Funding

The study was supported by Indian Council of Agricultural Research, New Delhi, India by grant in aid through ICAR-CPCRI Institutional Project (1000765041).

### Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

### Consent for publication

All authors consent to the publication of this manuscript in Crop Protection.

### CRedit authorship contribution statement

**Shivaji Hausrao Thube:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **R Thava Prakasa Pandian:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **M. Rajkumar:** Writing – review & editing, Formal analysis, Data curation. **Merin Babu:** Writing – review & editing, Supervision, Investigation, Formal analysis, Conceptualization. **A. Josephraj Kumar:** Writing – review & editing, Supervision, Resources, Formal analysis, Conceptualization. **Santhoshkumar P:** Methodology, Investigation, Data curation. **B.J. Nirmal Kumar:** Writing – review & editing, Methodology, Investigation, Data curation. **Vinayaka Hegde:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Conceptualization. **Balanagouda Patil:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **H. Rajashekara:** Writing – review & editing, Methodology, Investigation, Formal analysis. **T. Prabhulinga:** Writing – review & editing, Software, Formal analysis. **Babasaheb B. Fand:** Writing – review & editing, Writing – original draft, Software, Formal analysis. **Shailesh Gawande:** Writing – review & editing, Writing – original draft, Software, Formal analysis. **Dipak Nagrale:** Writing – review & editing, Writing – original draft, Software, Formal analysis. **M. Devindrappa:** Writing – review & editing, Writing – original draft, Software, Formal analysis. **M.K. Rajesh:** Writing – review & editing, Writing – original draft, Supervision, Software, Formal analysis.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper entitled “*Euwallacea perbrevis* (Eichhoff, 1868) and associated novel fungal symbiont, *Fusarium* sp.: A potential cause of wilting in cocoa, *Theobroma cacao* in India”.

### Data availability

Data will be made available on request.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cropro.2024.106754>.

### References

- Akbar, A., Hussain, S., Ullah, K., Fahim, M., Ali, G.S., 2018. Detection, virulence and genetic diversity of *Fusarium* species infecting tomato in Northern Pakistan. *PLoS One* 13, e0203613. <https://doi.org/10.1371/journal.pone.0203613>.
- Bateman, C., Sigut, M., Skelton, J., Smith, K.E., Hulcr, J., 2016. Fungal associates of the *Xylosandrus compactus* (Coleoptera: Curculionidae, Scolytinae) are spatially segregated on the insect body. *Environ. Entomol.* 45, 883–890. <https://doi.org/10.1093/ee/nvw070>.
- Batra, L.R., 1963. Ecology of ambrosia fungi and their dissemination by beetles. *Trans. Kans. Acad. Sci.* 66, 213–236. <https://doi.org/10.2307/3626562>.
- Beaver, R.A., 1989. Insect–fungus relationships in the bark and ambrosia beetles. In: Wilding, N., Collins, N.M., Hammond, P.M., Webber, J.F. (Eds.), *Insect–fungus Interactions*. Academic Press, London, UK, pp. 121–143. <https://doi.org/10.1016/B978-0-12-751800-8.50011-2>.
- Biedermann, P.H.W., Vega, F.E., 2020. Ecology and evolution of insect–fungus mutualisms. *Annu. Rev. Entomol.* 65, 431–455.
- Booth, C., 1971. *The genus Fusarium*. Commonwealth Mycological Institute, Surrey, England, p. 237..
- Bosso, L., Senatore, M., Varlese, R., Ruocco, M., Garonna, A.P., Bonanomi, G., Mazzoleni, S., Cristinzio, G., 2012. Severe outbreak of *Fusarium solani* on *Quercus ilex* vectored by *Xylosandrus compactus*. *J. Plant Pathol.* 94, S4–S99. <https://doi.org/10.4454/JPP.V95I4SUP.036>.
- Bowen, K.L., Hagan, A.K., Pegues, M., Jones, J., Miller, H.B., 2018. Epidemics and yield losses due to *Corynespora cassicola* on cotton. *Plant Dis.* 102, 2494–2499. <https://doi.org/10.1094/PDIS-03-18-0382-RE>.
- Brayford, D., 1987. *Fusarium bungicourtii* sp. nov. and its relationship to *F. tumidum* and *F. tumidum* var. *coeruleum*. *Trans. Br. Mycol. Soc.* 89, 347–351. [https://doi.org/10.1016/S0007-1536\(87\)80117-1](https://doi.org/10.1016/S0007-1536(87)80117-1).
- Carreras-Villasenor, N., Rodríguez-Haas, J.B., Martínez-Rodríguez, L.A., Pérez-Lira, A.J., Ibarra-Laclette, E., Villafan, E., Castillo-Díaz, A.P., Ibarra-Juarez, L.A., Carrillo-Hernández, E.D., Sánchez-Rangel, D., 2022. Characterization of two *Fusarium solani* species complex isolates from the ambrosia beetle *Xylosandrus morigerus*. *J. Fungi* 8, 231. <https://doi.org/10.3390/jof8030231>.
- Carrillo, D., Cruz, L., Kendra, P., Narvaez, T., Montgomery, W., Monterroso, A., De Grave, C., Cooperband, M., 2016. Distribution, pest status, and fungal associates of *Euwallacea nr. formicatus* in Florida avocado groves. *Insects* 7, 55. <https://doi.org/10.3390/insects7040055>.
- Ceriani-Nakamurakare, E., Robles, C., González-Audino, P., Dolinko, A., Mc Cargo, P., Corley, J., Allison, J., Carmarán, C., 2022. The ambrosia beetle, *megaplatypus mutatus*: a threat to global broad-leaved forest Resources. *J. Integr. Pest Manag.* 13, 21. <https://doi.org/10.1093/jipm/pmac016>.
- DCCD, 2020. Cocoa area, production, and productivity for the year 2019–20. Retrieved from. <https://dccd.gov.in/Content.aspx?mid=1072&tid=1>.
- Eskalen, A., Gonzalez, A., Wang, D.H., Twizeyimana, M., Mayorquin, S.J., Lynch, S.C., 2012. First report of a *Fusarium* sp. and its vector tea shot hole borer (*Euwallacea formicatus*) causing *Fusarium dieback* on avocado in California. *Plant Dis.* 96, 1070. <https://doi.org/10.1094/PDIS-03-12-0276-PDN>.
- Eskalen, A., Stouthamer, R., Lynch, S.C., Rugman-Jones, P.F., Twizeyimana, M., Gonzalez, A., Thibault, T., 2013. Host range of *Fusarium dieback* and its ambrosia beetle (Coleoptera: Scolytinae) vector in southern California. *Plant Dis.* 97, 938–951. <https://doi.org/10.1094/PDIS-11-12-1026-RE>.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3, 294–299.
- Freeman, S., Sharon, M., Maymon, M., Mendel, Z., Protasov, A., Aoki, T., Eskalen, A., O'Donnell, K., 2013. *Fusarium euwallaceae* sp. nov. - a symbiotic fungus of *Euwallacea* sp., an invasive ambrosia beetle in Israel and California. *Mycologia* 105, 1595–1606. <https://doi.org/10.3852/13-066>.
- Freeman, S., Sharon, M., Dori-Bachash, M., Maymon, M., Belasov, E., Maoz, Y., Margalit, O., Protasov, A., Mendel, Z., 2016. Symbiotic association of three fungal

- species throughout the life cycle of the ambrosia beetle *Euwallacea* nr. *Fornicatus*. *Symb.* 68, 115–128. [10.1007/s13199-015-0356-9](https://doi.org/10.1007/s13199-015-0356-9).
- García-Avila, C.D.J., Trujillo-Arriaga, F.J., López-Buenfil, J.A., González-Gómez, R., Carrillo, D., Cruz, L.F., Ruiz-Galván, I., Quezada-Salinas, A., Acevedo-Reyes, N., 2016. First report of *Euwallacea* nr. *fornicatus* (Coleoptera: Curculionidae) in Mexico. *Fla. Entomol.* 99, 555–556. <https://doi.org/10.1653/024.099.0335>.
- Gomez, D.F., Rabaglia, R.J., Fairbanks, K.O., Hulcr, J., 2018. North American Xyleborini north of Mexico: a review and key to genera and species (Coleoptera, Curculionidae, Scolytinae). *ZooKeys* 768, 19–68. <https://doi.org/10.3897/zookeys.768.24697>.
- Haack, R.A., Rabaglia, R.J., 2013. Exotic bark and ambrosia beetles in the USA: potential and current invaders. In: Pena, Jorge E. (Ed.), *Potential Invasive Pests of Agricultural Crops*. CAB International, Wallingford, UK, pp. 48–74. <https://doi.org/10.1079/9781845938291.0048>.
- Harrington, T.C., McNew, D., Mayers, C., Fraedrich, S.W., Reed, S.E., 2014. *Ambrosiella roeperi* sp. nov. is the mycangial symbiont of the granulate ambrosia beetle, *Xylosandrus crassiusculus*. *Mycologia* 106, 835–845. <https://doi.org/10.3852/13-354>.
- Hughes, M.A., 2013. *The Evaluation of Natural Resistance to Laurel Wilt Disease in Redbay (Persea borbonia)*. Univ. Florida, Dissertation. Gainesville.
- Hulcr, J., Dunn, R., 2011. The sudden emergence of pathogenicity in insect-fungus symbioses threatens naive forest ecosystems. *Proc. Royal Soc. B* 278, 2866–2873. <https://doi.org/10.1098/rspb.2011.1130>.
- Hulcr, J., Stelinski, L.L., 2017. The ambrosia symbiosis: from evolutionary ecology to practical management. *Annu. Rev. Entomol.* 62, 285–303. <https://doi.org/10.1146/annurev-ento-031616-035105>.
- Hulcr, J., Black, A., Prior, K., Chen, C.-Y., Li, H.-F., 2017. Studies of ambrosia beetles (Coleoptera: Curculionidae) in their native ranges help predict invasion impact. *Fla. Entomol.* 100, 257–261. <https://doi.org/10.1653/024.100.0219>.
- Hulcr, J., Atkinson, T.H., Cognato, A.I., Jordal, B.H., McKenna, D.D., O'Brien, C.W., 2019. Morphology, taxonomy, and phylogenetics of bark beetles. In: *Bark Beetles*. Academic Press, pp. 41–84.
- Kasson, M.T., O'Donnell, K., Rooney, A.P., Sink, S., Ploetz, R.C., Ploetz, J.N., Konkol, J. L., Carrillo, D., Freeman, S., Mendel, Z., Smith, J.A., Black, A.W., Hulcr, J., Bateman, C., Stefkova, K., Campbell, P.R., Geering, A.D.W., Dann, E.K., Eskalen, A., Mohotti, K., Short, D.P.G., Aoki, T., Fenstermacher, K.A., Davis, D.D., Geiser, D.M., 2013. An inordinate fondness for *Fusarium*: phylogenetic diversity of fusaria cultivated by ambrosia beetles in the genus *Euwallacea* on avocado and other plant hosts. *Fungal Genet. Biol.* 56, 147–157. <https://doi.org/10.1016/j.fgb.2013.04.004>.
- Kühnholz, S., Borden, J.H., Uzunovic, A., 2001. Secondary ambrosia beetles in apparently healthy trees: adaptations, potential causes, and suggested research. *Integrated Pest Manag. Rev.* 6, 209–219. <https://doi.org/10.1023/A:1025702930580>.
- Lahive, F., Hadley, P., Daymond, A.J., 2019. The physiological responses of cacao to the environment and the implications for climate change resilience. *A Rev. Agronomy Sustain. Develop.* 39, 5. <https://doi.org/10.1007/s13593-018-0552-0>.
- Lynch, S.C., Twizeyimana, M., Mayorquin, J.S., Wang, D.H., Na, F., Kayim, M., Hulcr, J., 2016. Identification, pathogenicity, and abundance of *Paracrematium pembeum* sp. nov. and *Graphium euwallaceae* sp. nov.—two newly discovered mycangial associates of the polyphagous shot hole borer (*Euwallacea* sp.) in California. *Mycologia* 108, 313–329. <https://doi.org/10.3852/15-063>.
- Marini, L., Haack, R.A., Rabaglia, R.J., Toffolo, E.P., Battisti, A., Faccoli, M., 2011. Exploring associations between international trade and environmental factors with establishment patterns of exotic Scolytinae. *Biol. Invasions* 13, 2275–2288. <https://doi.org/10.1007/s10530-011-0039-2>.
- Mendel, Z., Protasov, A., Sharon, M., Zveibil, A., Yehuda, S., O'Donnell, K., Rabaglia, R., Wysoki, M., Freeman, S., 2012. An Asian ambrosia beetle *Euwallacea fornicatus* and its novel symbiotic fungus *Fusarium* sp. pose a serious threat to the Israeli avocado industry. *Phytoparasitica* 40, 235–238. <https://doi.org/10.1007/s12600-012-0223-7>.
- Morales-Rodríguez, C., Sferrazza, I., Aleandri, M.P., Dalla Valle, M., Speranza, S., Contarini, M., Vannini, A., 2021. The fungal community associated with the ambrosia beetle *Xylosandrus compactus* invading the mediterranean maquis in central Italy reveals high biodiversity and suggests environmental acquisitions. *Fungal Biol.* 125, 12–24. <https://doi.org/10.1016/j.funbio.2020.09.008>.
- Na, F., Carrillo, J.D., Mayorquin, J.S., Ndinga-Muniania, C., Stajich, J.E., Stouthamer, R., Huang, Y.T., Lin, Y.T., Chen, C.U., Eskalen, A., 2018. Two novel fungal symbionts *Fusarium kuroshium* sp. nov. and *Graphium kuroshium* sp. nov. Of Kuroshio shot hole borer (*Euwallacea* sp. nr. *fornicatus*) cause *Fusarium* dieback on woody host species in California. *Plant Dis.* 102, 1154–1164. <https://doi.org/10.1094/PDIS-07-17-1042-RE>.
- Nelson, P.E., Tousson, T.A., Marasas, W.F.O., 1983. *Fusarium* Species: an Illustrated Manual for Identification. University Park, Pennsylvania State, p. 193. Univ Press.
- O'Donnell, K., 2000. Molecular phylogeny of the *Nectria Haematococca-Fusarium Solani* species complex. *Mycol.* 92, 919–938. <https://doi.org/10.1080/00275514.2000.12061237>.
- O'Donnell, K., Sink, S., Libeskind-Hadas, R., Hulcr, J., Kasson, M.T., Ploetz, R.C., Konkol, J.L., Ploetz, J.N., Carrillo, D., Campbell, A., Duncan, R.E., Liyanage, P.N., Eskalen, A., Na, F., Geiser, D.M., Bateman, C., Freeman, S., Mendel, Z., Sharon, M., Aoki, T., Crosse, A.A., Rooney, A.P., 2015. Discordant phylogenies suggest repeated host shifts in the *Fusarium-Euwallacea* ambrosia beetle mutualism. *Fungal Genet. Biol.* 82, 277–290. <https://doi.org/10.1016/j.fgb.2014.10.014>.
- Ploetz, R.C., Hulcr, J., Wingfield, M.J., deBeer, Z.W., 2013. Destructive tree diseases associated with ambrosia and bark beetles: black swan events in tree pathology. *Plant Dis.* 95, 856–872. <https://doi.org/10.1094/PDIS-01-13-0056-FE>.
- Paap, T., De Beer, Z.W., Migliorini, D., Nel, W.J., Wingfield, M.J., 2018. The polyphagous shot hole borer (PSHB) and its fungal symbiont *Fusarium euwallaceae*: a new invasion in South Africa. *Australas. Plant Pathol.* 47, 231–237. <https://doi.org/10.1007/s13313-018-0545-0>.
- Pandian, R.T.P., Thube, S.H., Merin, B., Pratibha, V.H., Rajkumar, M., Mhatre, P.H., Hegde, V., 2022. First record of *Fusarium falciforme* (FSSC 3+4) a relevant human pathogen causing root decay of arecanut, *Areca catechu* L. *J. Plant Dis. Prot.* 129, 695–699. <https://doi.org/10.1007/s41348-022-00581-z>.
- Prior, C., 1986. Sudden death of cocoa in Papua New Guinea associated with *Phytophthora palmivora* cankers invaded by bark beetles. *Ann. Appl. Biol.* 109, 535–543. <https://doi.org/10.1111/j.1744-7348.1986.tb03210.x>.
- Ranger, C.M., Reding, M.E., Schultz, P.B., Oliver, J.B., 2012. Ambrosia beetle (Coleoptera: Curculionidae) responses to volatile emissions associated with ethanol-injected *Magnolia virginiana*. *Environ. Entomol.* 41, 636–647. <https://doi.org/10.1603/EN11299>.
- Ranger, C.M., Reding, M.E., Schultz, P.B., Oliver, J.B., 2013. Influence of flood-stress on ambrosia beetle host-selection and implications for their management in a changing climate. *Agric. For. Entomol.* 15, 56–64.
- Ratnam, R., 1961. Introduction of criollo cocoa into madras state. *South Indian Hortic.* 9, 24–29.
- Rosmana, A., Hikmawati, H., Asman, A., 2013. Identification of a disease on cocoa caused by *Fusarium* in Sulawesi. *Pelita Perkebunan (Coffee Cocoa Res. J.)* 29, 210–219. <https://doi.org/10.22302/iccr.jur.pelita.perkebunan.v29i3.13>.
- Ruzzier, E., Ortis, G., Vallotto, D., Faccoli, M., Martínez-Sanudo, I., Marchioro, M., 2023. The first full host plant dataset of Curculionidae Scolytinae of the world: tribe Xyleborini LeConte, 1876. *Sci. Data* 10, 166. <https://doi.org/10.1038/s41597-023-02083-5>.
- Sittichaya, W., Permkam, S., Cognato, A.I., 2012. Species composition and flight pattern of Xyleborini ambrosia beetles (Col.: Curculionidae: Scolytinae) from agricultural areas in southern Thailand. *Environ. Entomol.* 41, 776–784. <https://doi.org/10.1603/EN11271>.
- Smith, S.M., Gomez, D.F., Hulcr, J., Cognato, A.I., 2019. Reassessment of the species in the *Euwallacea fornicatus* (Coleoptera: Curculionidae: Scolytinae) complex after the rediscovery of the “lost” type specimen. *Insects* 10 (9), 261.
- Stouthamer, R., Jones, P.R., Thu, P.Q., Eskalen, A., Thibault, T., Hulcr, J., Wang, L.J., Jordal, B.H., Chen, C.Y., Cooperband, M., Lin, C.S., Kamata, N., Lu, S.S., Masuya, H., Mendel, Z., Rabaglia, R., Sanguansub, S., Shih, H.H., Sittichaya, W., Zong, S., 2017. Tracing the origin of a cryptic invader: phylogeography of the *Euwallacea fornicatus* (Coleoptera: Curculionidae: Scolytinae) species complex. *Agric. For. Entomol.* 19, 366–375. <https://doi.org/10.1111/afe.12215>.
- Tantaoui, A., Ouintin, M., Geiger, J.-P., Fernandez, D., 1996. Characterization of a single clonal lineage of *Fusarium oxysporum* f. sp. *abedinis* causing Bayoud disease of date palm in Morocco. *Phytopathology* 86, 787–792. <https://doi.org/10.1094/PHYTO-86-787>.
- Thube, S.H., Mahapatro, G.K., Mohan, C., Pandian, R.T.P., Apshara, E., Jose, C.T., 2019. Biology, feeding, and oviposition preference of *Helopeltis theivora*, with notes on the differential distribution of species of the tea mosquito bug species complex across elevations. *Anim. Biol. Leiden* 70, 67–79. <https://doi.org/10.1163/15707563-20191083>.
- Thube, S.H., Pandian, R.T.P., Josephraj Kumar, A., Bhavishya, A., Nirmal Kumar, B.J., Firake, D.M., Shah, V., Madhu, T.N., Ruzzier, E., 2022a. *Xylosandrus crassiusculus* (motschulsky) on cocoa pods (*Theobroma cacao* L.): matter of bugs and fungi. *Insects* 13, 809. <https://doi.org/10.3390/insects13090809>.
- Thube, S.H., Pandian, R.T.P., Bhavishya, A., Babu, M., Josephraj Kumar, A., Chaithra, M., Hegde, V., Ruzzier, E., 2022b. *Xylosandrus crassiusculus* (Motschulsky) (Coleoptera: Curculionidae) and its fungal symbiont *Ambrosiella roeperi* associated with arecanut kernel decay in Karnataka, India. *Insects* 13, 1–12. <https://doi.org/10.3390/insects13010067>.
- Vannini, A., Contarini, M., Faccoli, M., Dalla Valle, M., Rodriguez, C.M., Mazzetto, T., Guarneri, D., Vettraino, A.M., Speranza, S., 2017. First report of the ambrosia beetle *Xylosandrus compactus* and associated fungi in the Mediterranean maquis in Italy, and new host-pest associations. *EPPO Bull.* 47, 100–103. <https://doi.org/10.1111/epp.12358>.
- White, T.J., Bruns, T., Lee, S.J.W.T., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: Guide Method. Applic.* 18, 315–322.
- Wingfield, M., Hammerbacher, A., Ganley, R., Steenkamp, E., Gordon, T., Wingfield, B., Coutinho, T., 2008. Pitch canker caused by *Fusarium circinatum* a growing threat to pine plantations and forests worldwide. *Australas. Plant Pathol.* 37, 319–334. <https://doi.org/10.1071/AP08036>.